Applicants: Hunziker et al. U.S.S.N.: 09/546,269

Listing of Claims

The listing presented below reflects the pending claims in the instant application as of the mailing date of the final Office Action (*i.e.*, May 1, 2003). No amendments have been made herein to the pending claims, and this listing of claims has been provided solely for the Examiner's convenience.

1. 23. (Cancelled)

- 24. (Previously Amended) A method for the selection of keratinocyte precursor cells from the outer root sheath of hair for subsequent use in a composition for healing a skin defect, comprising the steps of:
 - (a) plucking of an anagen or growing hair;
 - (b) primary-culturing the outer root sheath-derived keratinocyte precursor cells by adhering said anagen hair, *in toto*, to a microporous membrane, which possesses growth-arrested limited feeder cells on its undersurface so as to select for keratinocyte precursor cells from the outer root sheath of hair, wherein the primary culture medium contains human serum in a concentration less than 5%;
 - (c) organotypically-culturing the outer root sheath cells harvested from said primary cultures by inoculating a microporous membrane which also possesses growth-arrested/limited feeder cells on its undersurface, wherein the organotypic culture medium contains human serum in a concentration less than 5% and the keratinocyte precursor cells are seeded at a density of between 3 x 10° cells cm² and 1 x 10° cells cm²;
 - (d) generating an epidermal or complex skin equivalent, for subsequent use as a graft insert, comprised of keratinocyte precursor cells by placing a carrier membrane on top of said organotypic-culture from step (c) and detaching said skin or epidermal equivalent, which is comprised of the keratinocyte precursor cells and carrier membrane, together as a single, laminar unit:
 - (e) contacting said epidermal or skin equivalent with a skin defect present on an individual, and immobilizing said epidermal or skin equivalent at the site of contact.

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25. (Original) The method of claim 24, wherein said outer root sheath cells are autologous cells derived from the individual who will subsequently undergo treatment for a skin defect.

- 26. (Original) The method of claim 24, wherein said outer root sheath cells are homologous cells.
- 27 (Cancelled)
- 28. (Original) The method of claim 24, wherein the culture density of said growth-arrested limited feeder cells on said microporous membrane is between about 1x10⁴ cells cm² and about 5x10⁴ cells cm².
- 29. (Original) The method of claim 24, wherein said growth-arrested limited feeder cells are banked or immortalized cells.
- 30. (Original) The method of claim 24, wherein said primary and organotypic cultures utilize autologous or homologous human serum.
- 31. (Cancelled)
- 32. (Original) The method of claim 24, wherein said epidermal or skin equivalent comprises outer root sheath cells cultured in a medium containing only homologous or autologous biological supplements.
- 33. (Original) The method of claim 24, wherein said epidermal or skin equivalent comprises outer root sheath cells cultured in a medium containing only homologous or autologous releasates from blood components.

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- (Original) The method of claims 24 or 33, wherein said epidermal or skin equivalent comprises outer root sheath cells cultured in a medium containing only homologous or autologous releasates from blood components at a concentration of about 0.1% to about 20%.
- 35. (Previously Amended) The method of claim 24 or 33, wherein said epidermal equivalents are coated on their top or cornified side with a fibrin glue.
- 36. (Original) The method of claims 24 or 35, wherein said epidermal equivalents are coated on their top or cornified side with a carrier membrane.
- 37. (Previously Amended) The method of claim 24, wherein said microporous membrane is coated by one or more extracellular matrix substances selected from a group consisting of: fibrin, fibronectin, collagens, laminins and hyaluronan.
- 38. (Original) The method of claims 24 or 37, wherein said microporous membrane possesses a growth-arrested limited feeder cell system on its undersurface with said feeder cells of at least one type of cell selected from the group comprising human dermal fibroblasts, epidermal cells, mesenchymal cells, neuronal cells and endothelial cells.
- 39. (Original) The method of claim 24, wherein said carrier membrane is made from one or more types of materials selected from the group comprising polyester. PTFE, polyurethane, hyaluronic acid, polylactic acid, collagen, or a silicone or vaseline gauze dressing.
- 40. (Original) The method of claim 24, wherein the size of said epidermal equivalent is selected from the group consisting of 1.0 cm, 1.5 cm, 2.0 cm, and 2.5 cm in diameter.